The Determination of Glucose by Photometry



Some Information about Biogas

- Biogas is a mixture of CH₄ (methane) and CO₂ (carbon dioxide)
- Produced anaerobically with several kinds of microorganisms
- The reaction equation of this process:
- $C_6H_{12}O_6 \longrightarrow 3 CO_2 + 3 CH_4$
- Why is glucose important?: C₆H₁₂O₆

 $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + Energy$

Because of the separation of the Glucose we can win Energy

Glucose



How do we activate the Glucose?



Glucose + ATP \longrightarrow G-6-P + ADP

Oxidation of Glucose-6-Phosphate



Principle of a Photometer



How and what does the Photometer measure? *Cuvette*



Curve of absorption by NAD⁺ and NADH



Different solutions for the lab work



- Blank to have a basic value
- Control to have a control value and find mistakes
- Four subject solutions to calculate an average

The experiment of determination of Glucose



Results of the determination of Glucose

Group number:		Mentor:		
	E1	E2	Result	
Blank				
Control				

<u>Sample 1 (S₁)</u>

Time:

S1	E1	E ₂	Result
1.1			
1.2			
1.3			
1.4			

Procedure for the determination of glucose 1) Blank: Measure the blank in the cuvette (B) with the green cap 1.1. pipette 500 µl buffer from the green falcon tube into the cuvette (B) 1.2. pipette 1000 µl distilled water, from the blue falcon tube into the cuvette (B) Measure the cuvette in the photometer = E1 2) Control: Measure in the cuvette (C) with the red cap 2.1 pipette 500 µl buffer into the cuvette (C)

2.2 pipette 50 μ glucose standard from the pink tube (C) into the cuvette

2.2 pipotto 050 ul distillad water into the swett

	Enzymatic glucose determination						
+	Pipette into the cuvette	Blank	Control	Sample			
	Buffer	500µl	500µl	500µl			
	"Sample"	50µl	50µl	50µІ			
	Distilled Water	950µl	950µl	950µl			
	Mix the solution by <u>suspending</u> and measure it in the photometer. Write down the result and call it E ₁						
	Enzymes	10µl	10µl	10µІ			
	Shake it again and wait 15 minutes, because the enzymes have time to react. After that measure it in the photometer and write down the result as: E2						

Procedure for the determination of glucose

1) Blank: Measure the blank in the cuvette (B) with the green cap

- pipette 500 µl buffer from the green falcon tube into the cuvette (B)
- pipette 1000 µl distilled water, from the blue falcon tube into the cuvette (B)



Measure the cuvette in the photometer = E_1

2) Control: Measure in the cuvette (C) with the red cap

- 2.1 pipette 500 µl buffer into the cuvette (C)
- 2.2 pipette 50 µl glucose standard from the pink tube (C) into the cuvette
- 2.3 pipette 950 µl distilled water into the cuvette



Measure the cuvette in the photometer = E1

3) Sample: Measure sample from the fermenter in the cuvettes 1-4

- 3.1 Centrifugation
- 3.1.1 give 1 ml sample in the tube (S1A)
- 3.1.2 take the tube with the sample into the centrifuge at 13.000 rpm for 5 minutes
- 3.1.3 pipette 200 µl from the supernatant into the second tube (\$1B)
- 3.1.4 take these tube into the centrifuge for 5 minutes at 13000 rpm

3.2 Dilution (1:100)

- 3.2.1 pipette 10 μ l from S₁₈ in each of the four tubes 1.1 1.4
- 3.2.2 pipette 990 μl distilled water from the blue falcon tube in the tubes 1.1 - 1.4
- 3.2.3 shake the tubes to mix the samples



Now you have 1:100 diluted sample

3.3 Measuring of the samples

- 3.3.1 fill out of the tubes 1.1 1.4, 50 μl diluted samples into each of the cuvettes 1 - 4
- 3.3.2 pipette 500 µl buffer from the green falcon tube into each of the cuvettes 1 - 4
- 3.3.3 pipette 950 µl distilled water into each of the cuvettes 1 4
- 3.3.4 turn the cuvette upside down to mixit



Measure the cuvettes (1 - 4) in the photometer = E1

4) Enzymes: Add to the cuvettes

- 4.1 pipette 10 µl of the enzymes out of the blue tube (E) into all of the six cuvettes (B, C, 1 - 4)
- 4.2 turn the cuvettes upside down to mix the enzymes with the sample
- 4.3 wait 15 minutes so that the enzymes have time to react



Measure the cuvettes (B, C, 1 - 4) in the photometer = E2 now you can calculate your final results on a PC

<u>Results of the determination of Glucose</u>

<u>Sample 3 (S₃)</u>

Group number:		Mentor:		Time:			
[F1	F2	Recult	S3	E1	Ez	Result
Blank			Result	3.1			
Control				3.2			
				3.3			
<u>Sample 1 (S₁)</u>				3.4			
		Tin	ne:	5.4			
					Average:		
S1	E1	Ez	Result				
1.1				Sample 4 (S ₄)			
1.2						Tim	ie:
1.3					1_	1	
1.4				S4	E1	Ez	Result
Average			4.1				
		Average.		4.2			
Sample 2 (S ₂) Time:			4.3				
			4.4				
					Average:		
Sz	E1	Ez	Result				
2.1				<u>Sample 5 (S₅)</u>			
2.2						Tim	ie:
2.3				Ss	E	E ₂	Result
2.4				5.1			
	•	Average:		0.1			

How to take Samples

- Take a sample from the fermenter with a syringe
- Put the sample into tubes and put them into a centrifuge for about 5 minutes
- Then you pipette 990µl distilled water and 10µl of the overcome from the sample into a tube. It's a dilution of 1:100
- > Now you have an almost clear sample



Enzymatic glucose determination



Thank you for your attention!